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Biological activities and phytochemicals of five orangutan food plants from Wehea-Kelay Landscape

A S A Andini[1], E T Arung[1,2], M Hendra[3], M D Suryadana[1], E Sudiono[4], M A Rifqi[4] and I W Kusuma[1,2,*]

[1] Faculty of Forestry, Mulawarman University, Samarinda 75119, East Kalimantan, Indonesia
[2] Collaborative Research Center for Biomass-Based Nanocosmetic, Mulawarman University, Samarinda 75119, East Kalimantan, Indonesia
[3] Faculty of Mathematic and Natural Sciences, Mulawarman University, Samarinda 75119, East Kalimantan, Indonesia
[4] Yayasan Konservasi Alam Nusantara, Samarinda, 75243, East Kalimantan, Indonesia

*E-mail address: kusuma_iw@yahoo.com

Abstract. Several studies on the therapeutic potential of primate plant food species have been reported. However, research into the medicinal properties of orangutan plant foods is still in its early stages. The purpose of this research is to examine the phytochemical composition and biological activities of five orangutan food plants taken from the Wehea-Kelay Landscape: *Aglaia elliptica*, *Croton argyratus*, *Artocarpus lanceifolius*, *Artocarpus odoratissimus*, and *Baccaurea macrocarpa*. Phytochemicals analysis were conducted by color changes and colorimetry methods. To determine the antioxidant activity, DPPH free radical scavenging was used. The agar-well diffusion technique was used to assay the antimicrobial activity against four pathogenic microorganisms. The results showed that the plants contained alkaloids, flavonoids, tannins, and carbohydrates. The total flavonoid content of the plants varied from 64.91 to 95.87 mg CE/g extract, whereas the total phenolic content ranged from 76.93 to 115.58 mg GAE/g extract. The antimicrobial assay revealed that at specific concentrations, the plants samples were able to inhibit the tested microorganisms. The five plants inhibited DPPH free radicals with IC_{50} values ranging from 47.22 to 77.6 ppm. The findings of this study demonstrated that the five plants possess antioxidant activity as scientific evidence of therapeutic properties of orangutan food plants.

1. Introduction

Orangutan are closely related to bonobos, chimpanzees, and gorillas with the characteristics of having a large body and brain size [1]. Orangutan are herbivorous animals that prefers to consume leaves and fruits due to the easier digestibility and higher nutritional content of these foods compared to other sources like piths and tree barks [2]. There have been over 1.000 species of plant, fungi and small animals included in the diet of orangutans. The main thing that is very important to know is the nutritional content of orangutan natural food in the forest which consists of carbohydrates, protein, fat, water, and tannins [3]. The nutritional composition of their diet significantly affects their growth,

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reproduction, and resilience against diseases [4]. Orangutan are capable of selecting their food for both nutritional needs and to treat health disorder they suffer [5]. Their food sources contain different chemical compounds, some of them contain active ingredients that they naturally use to treat themselves, it has been reported that orangutans chew medicinal plants and put them on the top of their bodies to treat themselves [6].

Orangutans have a relatively close genetic relationship with humans [7], so that research related to orangutan food and medicinal potential can become a reference material for the human health sector. This is proven by the previous research that stated the *Zingiberaceae* family, commonly known as ginger plants are used for orangutan self medication [8] and ginger plants are beneficial as nutritional source after childbirth [9]. The bark of the *Dracontomelon dao* Blanco, or the New Guinea Walnut tree, is employed to address diarrhea [10]. *Dracena cantleyi* is used by orangutans by chewing until they release white foam which is then rubbed on their body allegedly to soothe muscles and joints, after several tests it was found that this leaf has anti-inflammatory properties [6]. With this research and study of medical and phytochemical potential of orangutan food plant, including *Aglaia elliptica, Croton argyratus, Artocarpus lanceifolius, Artocarpus odoratissimus,* and *Baccaurea macrocarpa* in landscapes of Wehea-Kelay, are one of the efforts to expand information sources and exploration related to the results of phytochemical and bioactivity tests.

2. Materials and Methods

2.1. Location

Orangutan food plant samples were collected in the Wehea-Kelay Landscape, located in the Kutai Timur-Berau Regency of East Kalimantan. The research was tested at the Laboratory of Chemistry for Forest Products and Renewable Energy, Faculty of Forestry, Mulawarman University, Samarinda.

2.2. Material

The main materials used were leaves extracts of *Croton argyratus, Aglaia elliptica, Artocarpus lanceifolius, Artocarpus odoratissimus,* and *Baccaurea macrocarpa.* The chemicals used in this research included 1,1-diphenyl-2-picrylhydrazyl, ascorbic acid, catechin, aluminium chloride, sodium nitrite, sodium hydroxide, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, dimethyl sulfoxide, and glucose. The equipment utilized comprises a rotary evaporator, laminar air flow, and a UV-Visible spectrophotometer (UV mini-1240, Shimadzu, Japan).

2.3. Methods

2.3.1. Sample Preparation. Plant sample preparation involves the collecting and processing samples. The plant samples that have been picked were then selected and cleaned with running water, then enter the drying process. The dried plant samples were then finely ground or cut into suitable sizes.
2.3.2. Extraction. Maceration method with ethanol solvent was used after drying the samples for 2 x 24 hours on a shaker. The result was separated into filtrate and residue through filtration, and then the solvent was evaporated using rotary vacuum evaporator to obtain a concentrated extract. Once the sample had become sufficiently concentrated, it was transferred into vials and placed in oven at the temperature 40°C to dryness.

2.3.3. Qualitative Phytochemical. Alkaloid test. 5 ml of test solvent was mixed with 2 ml of HCL, then 1 ml of Dragendorff solvent was added. If the color of the solvent changes to orange or red, it indicated the presence of alkaloids [11]. Flavonoid test. 1 ml of dissolved sample solution was added with a few drops of dilute sodium hydroxide 1% (NaOH). The flavonoid appearance was indicated if the solution turns into yellow and become colorless after hydrochloric acid 1% (HCl) addition [11]. Saponin test. The Saponins can identified by foam test in hot water. Stable foam that persists for 5 minutes and does not disappear upon the addition of 1 drop of HCl 2N, indicated the presence of saponins [12]. Tannin test. A test tube containing 1 ml of the sample solution was added with a few drops of 1% lead acetate solution $(CH_3COOH)_2Pb$. If the color shift into yellow precipitate indicates positive in tannin [11]. Triterpenoid and steroid test. 1 ml of the test sample mixed with 10 drops of acetic anhydride and 2

drops of concentrated sulfuric acid. Following with shaken the test samples and given a few minutes. A color change will appear as a result of the reaction. The test is positive for triterpenoid if red or purple coloration occur. A green or blue indicates a positive steroid test [13]. Carbohydrate test. One drop of molisch reagent is added to 1 ml of test sample, and then shaken. 1 ml of concentrated sulfuric acid is added down the side of test tube, if a purple ring forms between the two layers, the test is considered positive fort he presence of carbohydrates [13]

2.3.4. Total Phenolic Content. This test used the Folin-Ciocalteu reagent method and Gallic Acid Equivalent (GAE) as standard. As much as 0.10 ml extract solution was poured into a test tube, then 0.1 ml of DMSO, 0.4 ml of aquades, and 1.15 ml of 7.5% sodium carbonate (Na_2CO_3) were added dropwise into the test tube. The solution was incubated for 5 minutes. Then 0.25 ml of Follin-C 10% was dripped and incubated again for 60 minutes. The absorbance was determined using UV-VIS spectrophotometer 760 nm. The linear regression equation of the gallic acid was used to determine the total phenolic content [14].
2.3.5. *Total Flavonoid Content.* This test used the colorimetric method, using catechin reagent. It was prepared by dropping 0.10 ml of extract solution, 0.1 ml of DMSO, 0.6 ml of aquades, and 0.01 of 5% sodium nitrite ($NaNO_2$) into test tube. The mixture was incubated for 5 minutes. Furthermore, 0.1 aluminium chloride ($AlCl_3$) 10% was dripped and then incubated for 5 minutes. Drop 0.5 ml of sodium hydroxide (NaOH) 1 M into the test tube. Measure the absorbance of the catechin standard solution using UV-Vis spectrophotometer at wavelength of 510 nm. The linear regression equation of the catechin was used to determine the total flavonoid content [15].
2.3.6. Antioxidant Activity Assay. The DPPH method was utilized to conduct this test. The observed results were then compared with ascorbic acid as the positive control and DPPH as the negative control. The testing was then measured on UV-Vis spectrophotometer at wavelength of 517 nm. For the testing, 33 µl of the sample, 467 µl of ethanol, and 500 µl of a 60 µM DPPH solution (dissolved in ethanol) are added to a test tube. The sample mixture sufficient once the sample volüme reaches 1000 µl. The solvent was then able to incubate at room temperature for about 20 minutes under low light Antioxidant activity is determined through the decolorization of DPPH using a UV-VIS spectrophotometer with a wavelength of 517 nm [16]. The testing is conducted with varying concentrations of 6,25 ppm, 12,5 ppm, 25 ppm, and 50 ppm, and it is repeated three times. Antioxidant activity is calculated using the equation :

$$\text{DPPH Free Radical Scavenging \%} = \frac{DPPH\ Absorbance - Sample\ absorbance}{DPPH\ Absorbance} \times 100$$

2.3.7. Antimicrobial Activity Assay. This test is conducted using the Kirby-Bauer method, namely the agar well diffusion. The microbes used for this test are *Candida albicans fungi, Cutibacterium acnes, Staphylococcus aureus,* and *Salmonella typhi.* For antimicrobial analysis, positive control solvent is prepared by dissolving 2 mg of chloramphenicol in 4 ml of DMSO. For antifungal testing, miconazole nitrite 200 mg is used in 4 ml of DMSO as positive control [17]. Both are using DMSO as negative control. The well/zone method was made in solid agar that has been previously inoculated with cultured microbial for 24 hours. Wells were created based on the extract concentrations (500, 250, 125, and 62.5 µg/well), then the wells were filled with the extract to be tested. After incubation, bacterial growth was observed to determine the size of the inhibition zone around the wells. The relative inhibition activity was calculated using the formula:

$$\text{Relative Inhibitory Activity (\%)} = \left(\frac{\text{Diameter of sample inhibition (mm)}}{\text{Diameter of positive control inhibition (mm)}} \times 100\right)$$

3. Results and Discussion

3.1. Extract Yield. Ethanol maceration was selected due to its simple procedure, relatively low operational cost, and its ability to prevent damage to thermolabile or heat-sensitive active compounds [18]. Ethanol maceration of five orangutan food plants yielded the highest in *Baccaurea macrocarpa*

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leaves extract, followed by *Artocarpus lanceifolius, Artocarpus odoratissimus*, *Aglaia elliptica,* and the lowest extract yield was *Croton argyratus.* The variability in extract yields among the five samples was likely due to variation in plant species and growing environments, which affect plant' growth and impact the chemical composition of the compounds produced [19].

Table 1. Yield percentage of orangutan food plant samples

Plant Samples	Parts	Sample weight (g)	Extract weight (g)	Yield (%)
Aglaia elliptica	Leaves	10	0.435	4.35
Croton argyratus	Leaves	10	0.196	1.96
Artocarpus lanceifolius	Leaves	10	0.491	4.91
Artocarpus odoratissimus	Leaves	10	0.476	4.76
Baccaurea macrocarpa	Leaves	10	1.181	11.81

3.2. Phytochemical qualitative. The result can be seen in Table 2, where the leaves extract of Aglaia elliptica contains alkaloid compound, indicated by a golden yellow color; flavonoid, evidenced by the change in color of the solution to colorless; tannin, with the presence of yellow precipitate; steroid, resulting in a green color; carbohydrates, leading to a purple precipitate. However, no saponin and triterpenoid compounds were found. The same pattern was observed with the presence of five compounds (alkaloid, flavonoid, tannin, steroid, and carbohydrate) among the total of 7 compounds in the extract leaves of *Croton argyratus, Artocarpus lanceifolius,* and *Artocarpus odoratissimus.* Meanwhile, the leaves extract of Baccaurea macrocarpa, only contains 4 out of 7 total compounds detected, namely alkaloid, flavonoid, tannin, and carbohydrate. Based on the result, *Artocarpus odoratissimus* leaves extract has more intense color compared to the other plant samples. In the previous researched, it was mentioned that Species of *Artocarpus* contain secondary metabolites belonging to groups of compounds such as terpenoid, neolignans, and Diels-Alder adduct [20].

Table 2. Phytochemical screening on orangutan food plant samples

Secondary metabolite	Result				
	AE	CA	AL	AO	BM
Alkaloid	++	+	++	+++	++
Flavonoid	+	+	+	+	+
Tanin	+	+	++	+	+
Saponin	-	-	-	-	-
Steroid	+	+	+	++	-
Triterpenoid	-	-	-	-	-
Karbohidrat	+	+	++	+++	+++

3.3. Total phenolic content, total flavonoid content, and antioxidant activity. Phenols are compounds that possess the ability to neutralize radicals due to their hydroxyl group content. This vital plant component releases hydrogen atoms from its hydroxyl group, forming stable phenoxy radicals. These radicals have the potential to cause various degenerative diseases [21]. To determine Gallic Acid

Equivalent (GAE), a linear regression was applied to gallic acid calibration curve, resulting in y = 0.0276x – 0.0069, with coefficient of determination (R^2) = 0.9843.

Table 3. Total phenolic content of orangutan food plants samples

Samples	Average Absorbance	Regression	mg GAE/g Extract
Aglaia elliptica	0,022		104.71
Croton argyratus	0,014		76.93
Artocarpus lanceifolius	0,017	y = 0,0276x -0,0069 R² = 0,9843	86.59
Artocarpus odoratissimus	0,025		115.58
Baccaurea macrocarpa	0,023		109.54

The principle of determining the flavonoid content is based on formation of reaction between flavonoid and $AlCl_3$, resulting in yellow-colored complex. Addition of NaOH solution forms a peach-colored complex compound that can be measured the absorbance at a wavelength of 510 nm [22]. To determine the Catechin Equivalent (CE) for 5 samples, a linear regression was applied to the catechin calibration curve, resulting in the equation y = 0.0323x – 0.0053, with a coefficient of determination (R^2) of 0.9978.

Table 4. Total flavonoid content of orangutan food plants samples

Samples	Average Absorbance	Regression	mg CE/g Extract
Aglaia elliptica	0,022		84,52
Croton argyratus	0,016		64,91
Artocarpus lanceifolius	0,017	y = 0,0323x – 0,0053 R² = 0,9978	69,04
Artocarpus odoratissimus	0,026		95,87
Baccaurea macrocarpa	0,024		90,71

The antioxidant activity test with these orangutan food plants was conducted because antioxidants can be obtained either synthetically or naturally. Side effects may occur from synthetic antioxidants such as BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene), or TBHQ (Tertiary Butylated Hydroxy Quinone). Therefore, natural antioxidant compunds are needed for an alternative to be used as subtitutes for synthetic antioxidants. Fruits and plants with secondary metabolites in the form of flavonoids and phenolics that can act as free radical scavangers can provide a natural source of antioxidants [23]. By the test, it can be seen that *Artocarpus odoratissimus* leaves extract has the best IC_{50}, where the best antioxidant activity is indicated by the smallest IC_{50} value.

Table 5. The IC_{50} of antioxidant activity of five orangutan food plants samples.

Plant Samples	Percentage Inhibition DPPH (%)				IC_{50} (ppm)
	6,25 ppm	12,5 ppm	25 ppm	50 ppm	
Aglaia elliptica	8	11	17	24	77,6
Croton argyratus	7	13	21	33	89
Artocarpus lanceifolius	17	18	23	33	84,4
Artocarpus odoratissimus	33	36	44	50	47,2
Baccaurea macrocarpa	12	20	28	36	51

The order of the best antioxidant values obtained was in line with the order of phenolic and flavonoid content of the orangutan food plant samples, namely *Artocarpus odoratissimus, Baccaurea macrocarpa, Aglaia elliptica, Artocarpus lanceifolius,* and *Croton argyratus.* The phenolic and flavonoid compounds contribute linearly to antioxidant activity, meaning the higher their content, the better the antioxidant activity value [24]. The activity of phenolic compounds, such as flavanols and flavones, heavily depends on the quantity and location of hydroxyl (-OH) groups, which play a role in neutralizing free radicals. The ability of flavonoid to suppress free radicals is related to their ability to donate electrons. This is why there is a connection between the total phenolic, flavonoid content and antioxidant activity. The higher the total phenol and flavonoid content, the higher antioxidant's ability to donate electrons, thereby supressing the development of free radicals. Phenolic and flavonoid components are the key compounds in antioxidant roles [25].

3.4. Antimicrobial assay. This test was conducted on five orangutan food plants against isolate of fungus *Candida albicans*, and three bacterial strains, *Cutibacterium acnes*, *Staphylococcus aureus,* and *Salmonella typhi*. The selection of this method is due by filling each well with the extract concentration, osmolarity occurs more comprehensively and homogeneously, resulting in a higher and stronger extract concentration that effective to inhibits microbial growth [26].

Table 6. Antimicrobial activity of five orangutan food plant samples

Samples	Microbes	Inhibition Percentage (%)			
		500 µg/well	250 µg/well	125 µg/well	62,5 µg/well
Aglaia elliptica	*C. acnes*	0	0	0	0
	S. aureus	0	0	0	0
	S. typhi	0	0	0	0
	C. albicans	45	36	33	31
Croton argyratus	*C. acnes*	37	24	0	0
	S. aureus	0	0	0	0
	S. typhi	34	26	0	0
	C. albicans	47	44	40	36
Artocarpus lanceifolius	*C. acnes*	37	34	0	0
	S. aureus	30	0	0	0
	S. typhi	37	27	0	0
	C. albicans	48	45	39	36
Artocarpus odoratissimus	*C. acnes*	41	38	35	33
	S. aureus	27	24	0	0
	S. typhi	28	25	23	0
	C. albicans	45	42	37	35
Baccaurea macrocaroa	*C. acnes*	11	0	0	7,1
	S. aureus	0	0	0	0
	S. typhi	0	0	0	0
	C. albicans	36,7	35	0	0

The larger the inhibition zone diameter, the more active the test substance as an antibacterial agent, indicating that a greater microbial growth can be inhibited by the test substance. One of the factors that affect the activity of microbial is the antimicrobial properties [27]. Another factor is the relation between secondary metabolites and antimicrobial activity, compounds like flavonoids, terpenoids, polyphenols, including tannins and essential oil work together to form complex compounds that disrupt or even damage the cell membranes of the tested microbes [28].

4. Conclusion

Orangutan food plants include of *Croton argyratus, Aglaia elliptica, Artocarpus lanceifolius, Artocarpus odoratissimus,* and *Baccaurea macrocarpa* revealed by this study have medicinal potential can inhibit both free radical and microbial growth, where this provides information about medicines or remedies in the field of health and beauty. It is hoped that this research will encourage the sustainable protection of orangutan habitats.

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